

## METHODS FOR GENERATING DOUBLED HAPLOID PLANTS

### Abstract of the Disclosure

5 The present invention provides methods for generating doubled haploid and/or  
haploid plants from microspores. In a presently preferred embodiment of the methods  
of the present invention, plant material is selected that bears reproductive organs  
containing microspores at a developmental stage that is amenable to androgenic  
induction. The microspores are treated by contacting the selected plant material with  
water and subjecting the selected plant material to temperature stress, and optionally  
to nutrient stress. Preferably the selected plant material is contacted with an effective  
10 amount of a sporophytic development inducer and an effective amount of an auxin  
and/or a cell spindle inhibiting agent. Optionally, the selected plant material is  
contacted with an effective amount of a cytokinin and/or an effective amount of a  
gibberellin. The treated microspores are isolated, preferably by density centrifugation  
utilizing a solution of 0.3 M mannitol layered over a higher density solution of a sugar,  
15 preferably maltose. The isolated, treated microspores are then cultured in a liquid  
nutrient suspension medium supplemented with at least one plant ovary or with an  
aliquot of plant ovary conditioned medium, until the microspores develop into  
embryoids. The embryoids are transferred to a regeneration medium and incubated  
therein until the embryoids develop into plants. The resulting plants may be haploid  
20 or doubled haploid and may also be genetically transformed.